

## Photosynthesis and Respiration of Ferns in Relation to Their Habitat

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There are at least 500 species of ferns in Malaysia and Singapore, and these plants form a conspicuous part of the local flora. The reason for their abundance lies in the particularly favorable climate of this part of the world. Holttum (1954) has divided the ferns of Malaysia and Singapore into various classes according to the conditions under which they grow: terrestrial sun ferns, terrestrial shade ferns, epiphytes of sheltered places, epiphytes of exposed places, rock and river-bank ferns, aquatic ferns, and mountain ferns. The fact that ferns grow in a great diversity of habitats makes them very interesting plants for physiological and ecological studies.

Interest in the physiological studies of ferns has been centered mainly in the growth and development of fern gametophytes (Allsopp & Rao, 1969; Bloom & Nicols, 1972; Miller & Miller, 1961; Raghavan, 1965; Sobota & Partanen, 1966), spore germination (Raghavan, 1971), and the changes accompanying the conversion of aquatic to land forms of *Marsilea* (Allsopp, 1955; Gaudet, 1963, 1965). More recently, the role of light in the interrelated processes of morphogenesis and photosynthesis of fern gametophytes has also been investigated (Donaher & Partanen, 1971). However, very little work has been done on photosynthesis and respiration of fern fronds, particularly in relation to their adaptations to different habitats.

### PLANT MATERIALS

*Angiopteris evecta* Hoffm. was collected from Bukit Tamah Nature Reserve, Singapore. Plants were potted and grown in a shady place for two months before the fronds were used for experiments. Plants of *Marsilea crenata* Presl were grown with submerged roots in a large water tank in an open place. A layer of soil for the anchorage of the roots was laid on the floor of a tank. *Adiantum philippense* L. was grown in a semi-shady place. *Gleichenia linearis* (Burm.) Clarke, *Nephrolepis biserrata* (Swartz) Schott, *Drymoglossum piloselloides* Presl, *Davallia* sp.; and *Asplenium nidus* L. were collected from various terrestrial and epiphytic habitats around the University campus.

Fronds were cut just before the experiments and taken to the laboratory where the stipes were recut under water to prevent possible blockage of the xylem with air, thereby affecting transpiration. The stipes were then placed in water in a small polyethylene bag. Together they were transferred and placed in the central plane of a lucite photosynthesis chamber. The chamber was positioned vertically, and light was supplied from one side of the chamber. The set up for CO<sub>2</sub> gas exchange analysis using a Beckman infra-red CO<sub>2</sub> analyser was similar to those previously described (Wong & Hew, 1973).

A closed system was used to measure the CO<sub>2</sub> compensation point and to study the effect of CO<sub>2</sub> concentration on apparent photosynthesis, while an open sys-

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tem was used to determine the steady rate of apparent photosynthesis at various light intensities.

To isolate glycolic acid oxidase, fronds (2 gm) were cut and homogenized in a Waring blender. Isolation medium contained 0.05M Tricine buffer (pH 8) with polyvinyl pyrrolidone (0.5 gm) added. The homogenate was filtered through eight layers of cheese cloth and then centrifuged at  $6000 \times g$  for 1 min. The supernatant fluid was used as a crude enzyme extract. Oxygen uptake by glycolic acid oxidase was measured with a Clark type  $O_2$  electrode. (Wong & Hew, 1973). Reaction medium contained  $10^{-2}$  M Tricine buffer pH 8.  $\alpha$  HPMS (Hydroxy 1-2-pyridine methane sulfonic acid) was used to inhibit glycolic acid oxidase activities.

All measurements were repeated at least twice with different plants.

#### PHOTOSYNTHESIS, PHOTORESPIRATION, AND DARK RESPIRATION

For all the ferns studied except *Drymoglossum piloselloides*, an increase of light intensity increased the rates of apparent photosynthesis (Figs. 1-8). For *Gleichenia* and *Nephrolepis*, light saturation was reached about  $8 \times 10^4$  erg  $cm^{-2}$   $sec^{-1}$  with a rate of 4 to 6 mg  $CO_2$   $hr^{-1}$   $gm^{-1}$  fresh weight (Table 1). Fronds of *Adiantum* and *Marsilea* have apparent photosynthetic rates similar to those of terrestrial sun ferns. The only difference was that they saturated at  $4 \times 10^4$  erg  $cm^{-2}$   $sec^{-1}$ . Very low rates of apparent photosynthesis were obtained for *Asplenium* and *Angiopteris*. Apparent photosynthesis saturated at  $2 \times 10^4$  erg

TABLE 1. PHOTOSYNTHESIS, PHOTORESPIRATION AND RESPIRATION OF SOME COMMON FERNS.

Species	Exposure & Habitat	$P_{max}^1$	Dark Respiration <sup>1</sup>	Photorespiration <sup>1</sup>
<i>Gleichenia linearis</i>	Sun, terrestrial	5.0, 6.0	1.0, 0.8	1.8, 1.7
<i>Nephrolepis biserrata</i>	Sun, terrestrial	4.2, 4.2	1.0, 0.6	2.1, 1.9
<i>Adiantum philippense</i>	Shade, terrestrial	4.0, 5.2	0.5, 0.5	—
<i>Marsilea crenata</i>	Sun, aquatic	5.0, 5.0	0.5, 0.7	—
<i>Angiopteris evecta</i>	Shade, terrestrial	1.5, 1.5	0.2, 0.4	0.8, 1.0
<i>Asplenium nidus</i>	Shade, epiphytic	1.1, 1.1	0.2, 0.25	0.9, 0.8

<sup>1</sup>Two replicates; the values are given in mg of  $CO_2$  per hour per gm fresh weight.

$cm^{-2}$   $sec^{-1}$ . Light compensation points were in the region of 1 to  $4 \times 10^3$  erg  $cm^{-2}$   $sec^{-1}$  for all the ferns except *Drymoglossum*.

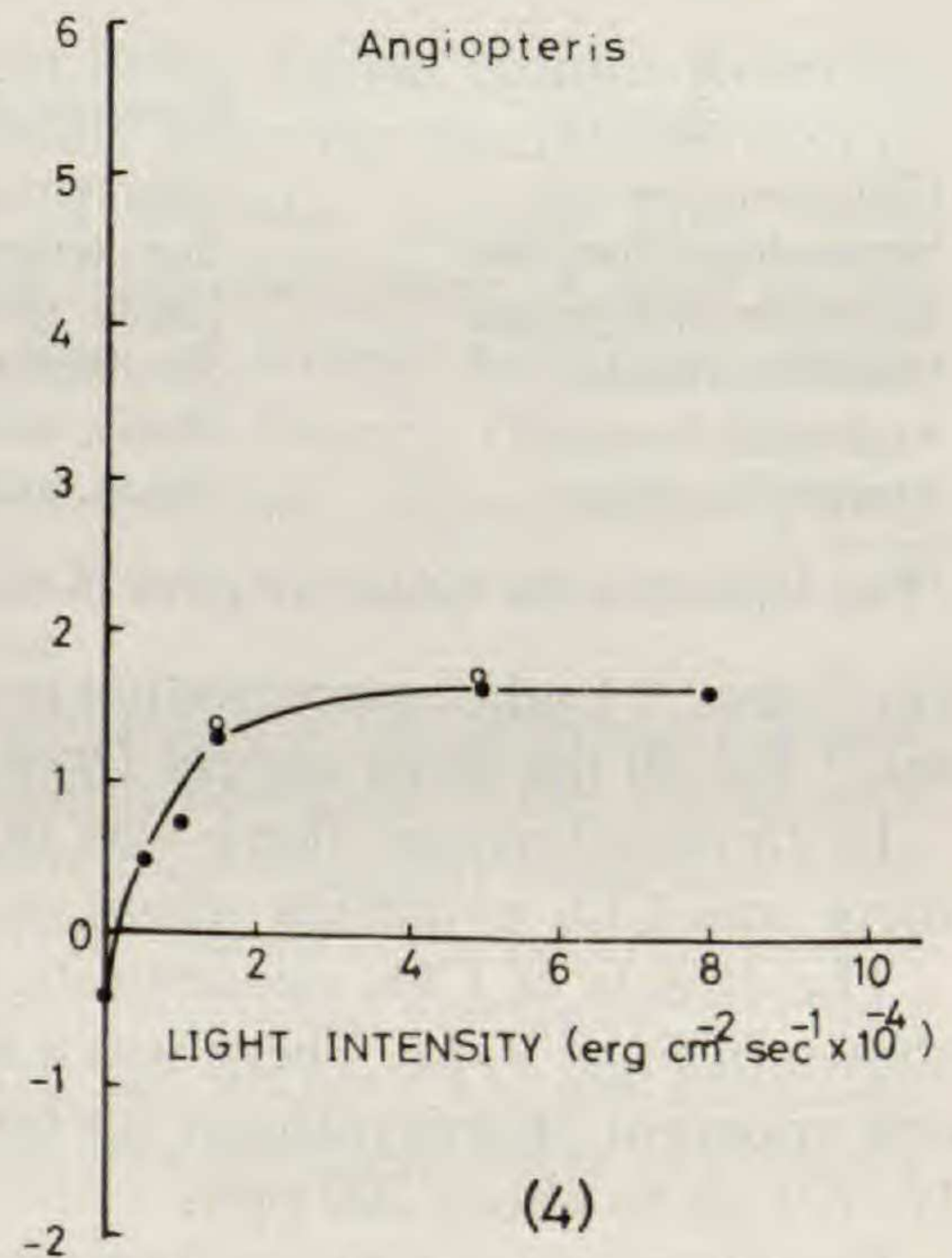
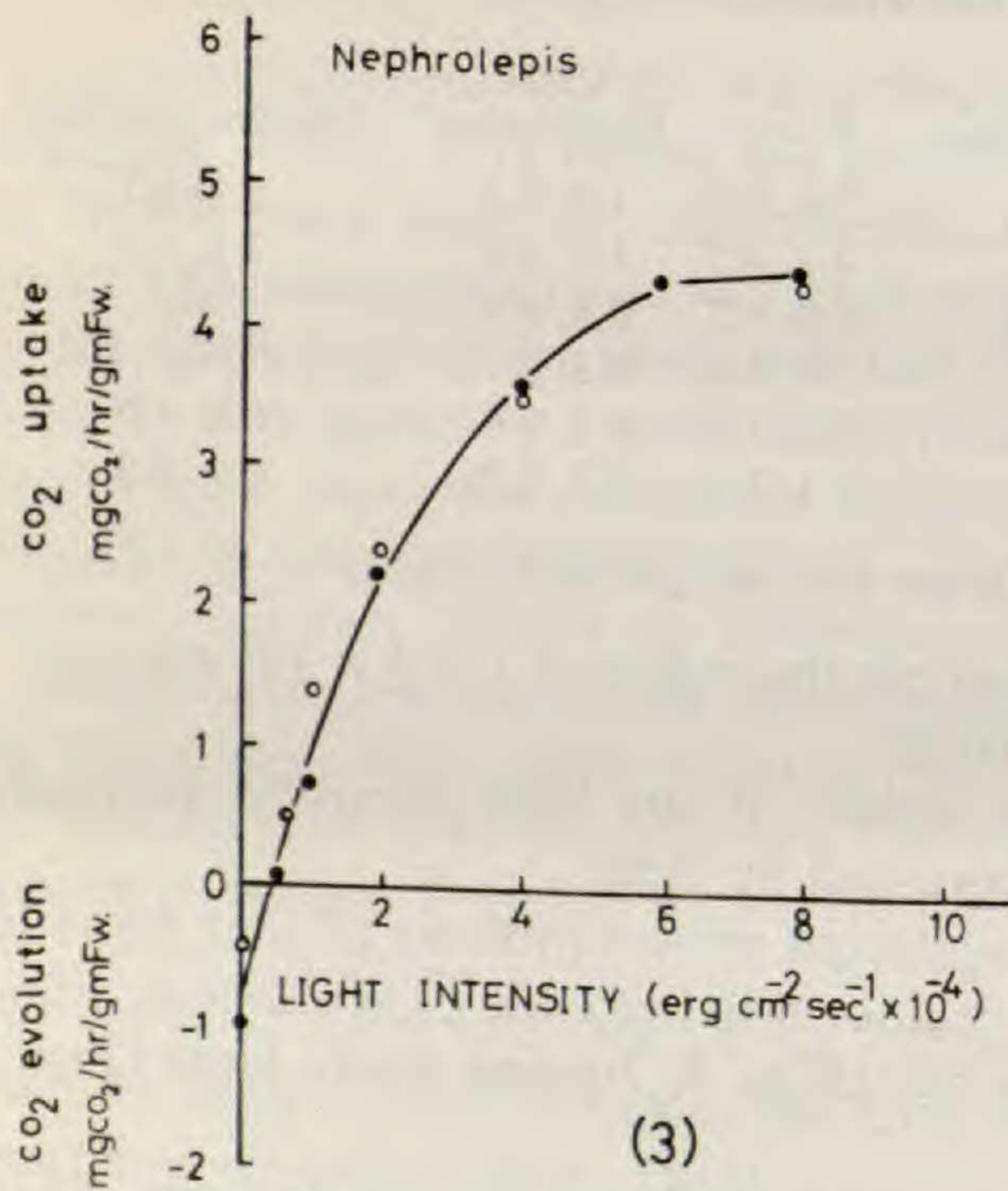
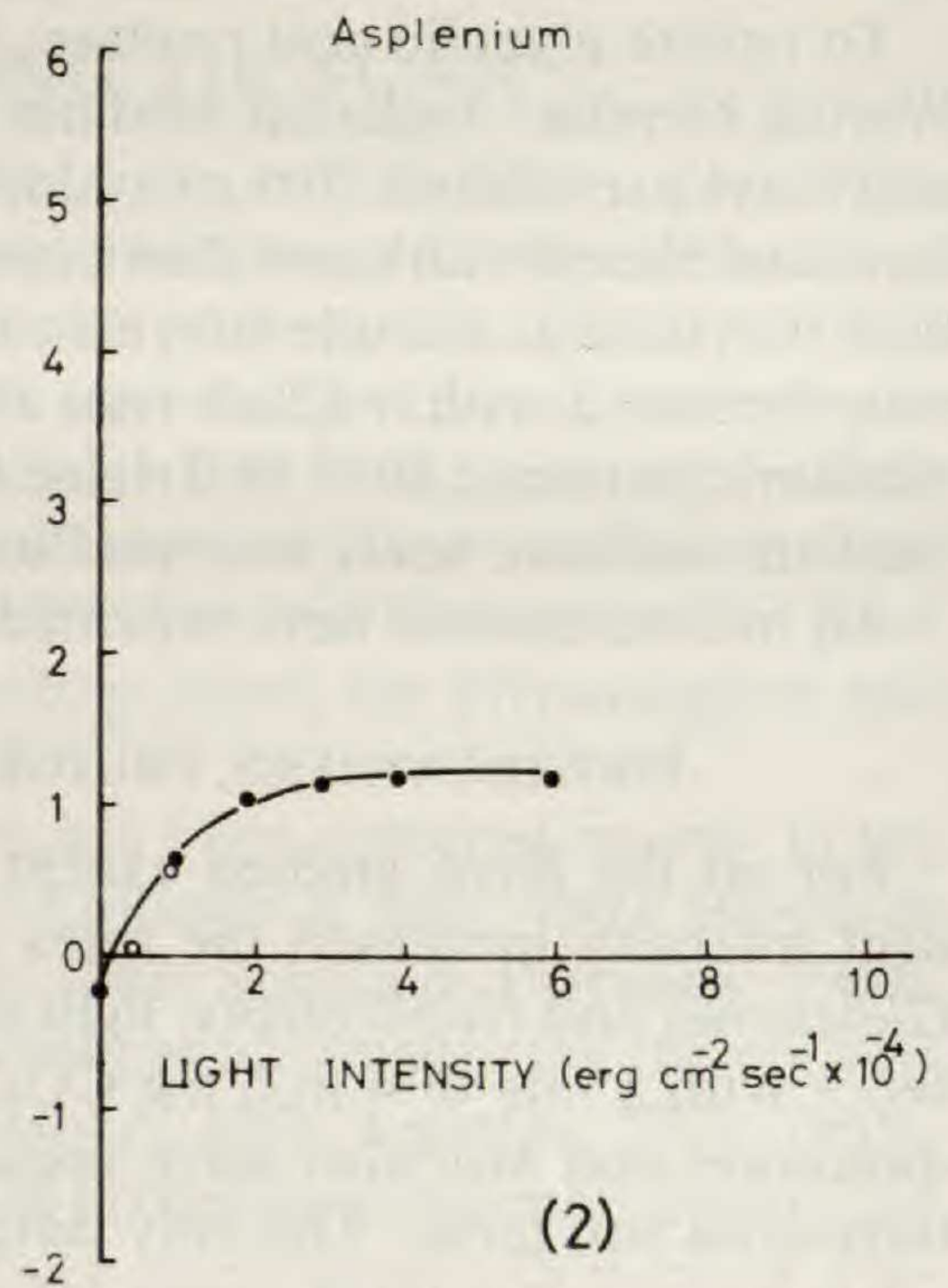
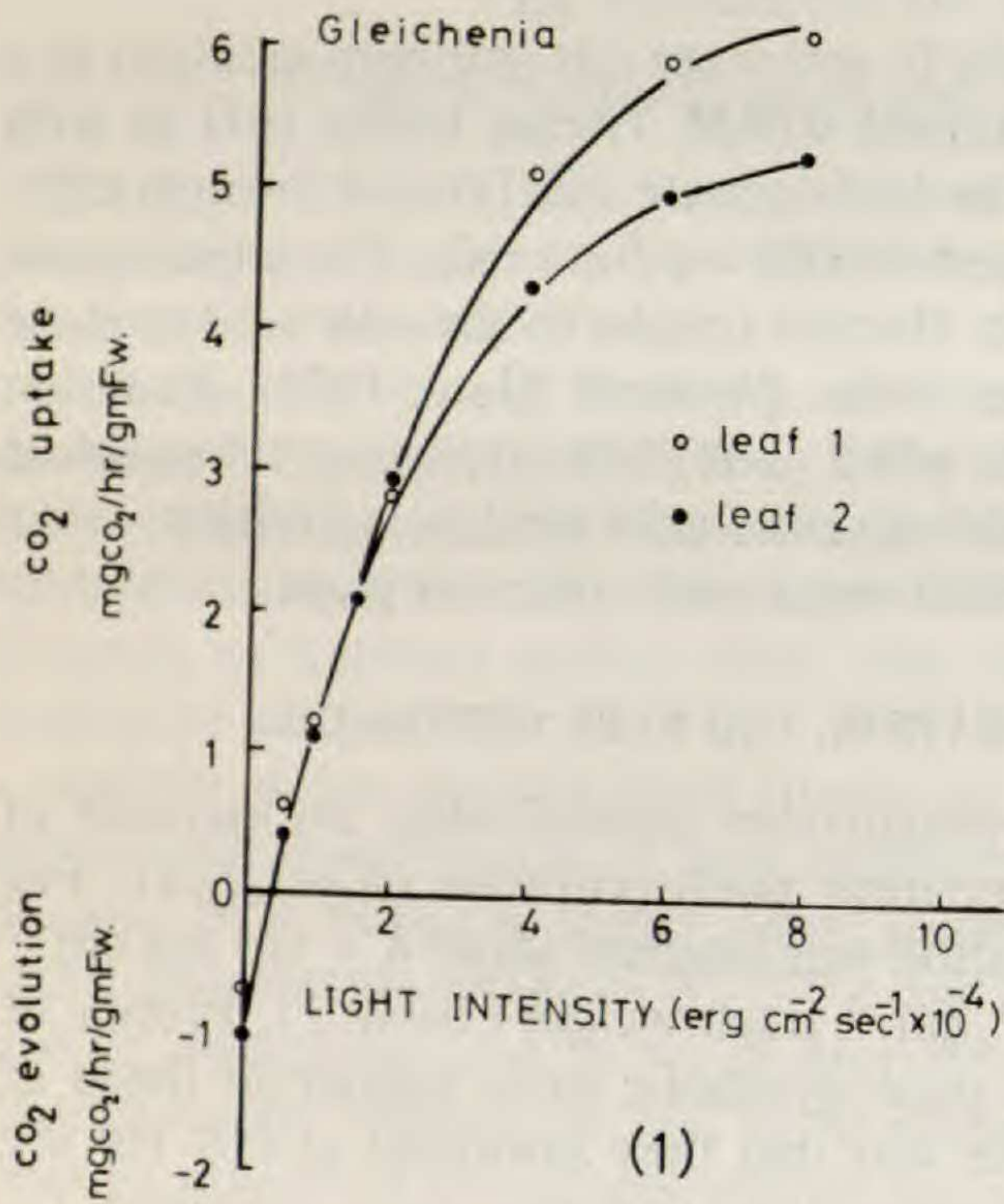
In *Drymoglossum*, there was no  $CO_2$  uptake at any light intensity. Instead, there was  $CO_2$  evolution which was suppressed by light.

The effects of  $CO_2$  concentration on apparent photosynthesis of four ferns is shown in Figs. 9-12. There was a linear relationship between  $CO_2$  concentration and apparent photosynthesis for both the sun (Figs. 9, 10) and shade ferns (Figs. 11, 12) up to about 200 ppm.

Postillumination  $CO_2$  outburst was observed in both sun and shade ferns. This was followed by a more steady but lower rate of dark respiration (Figs. 9-12).

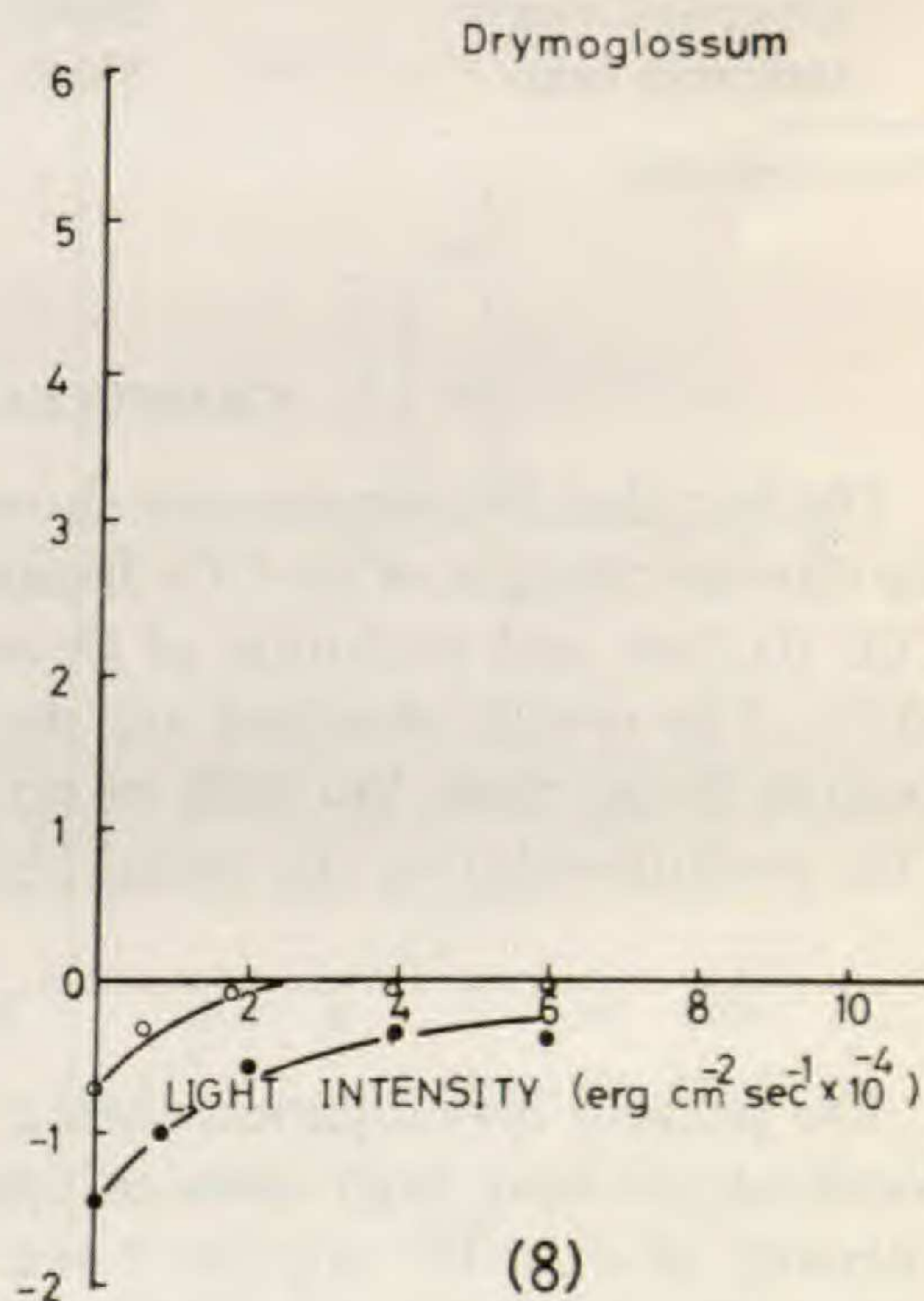
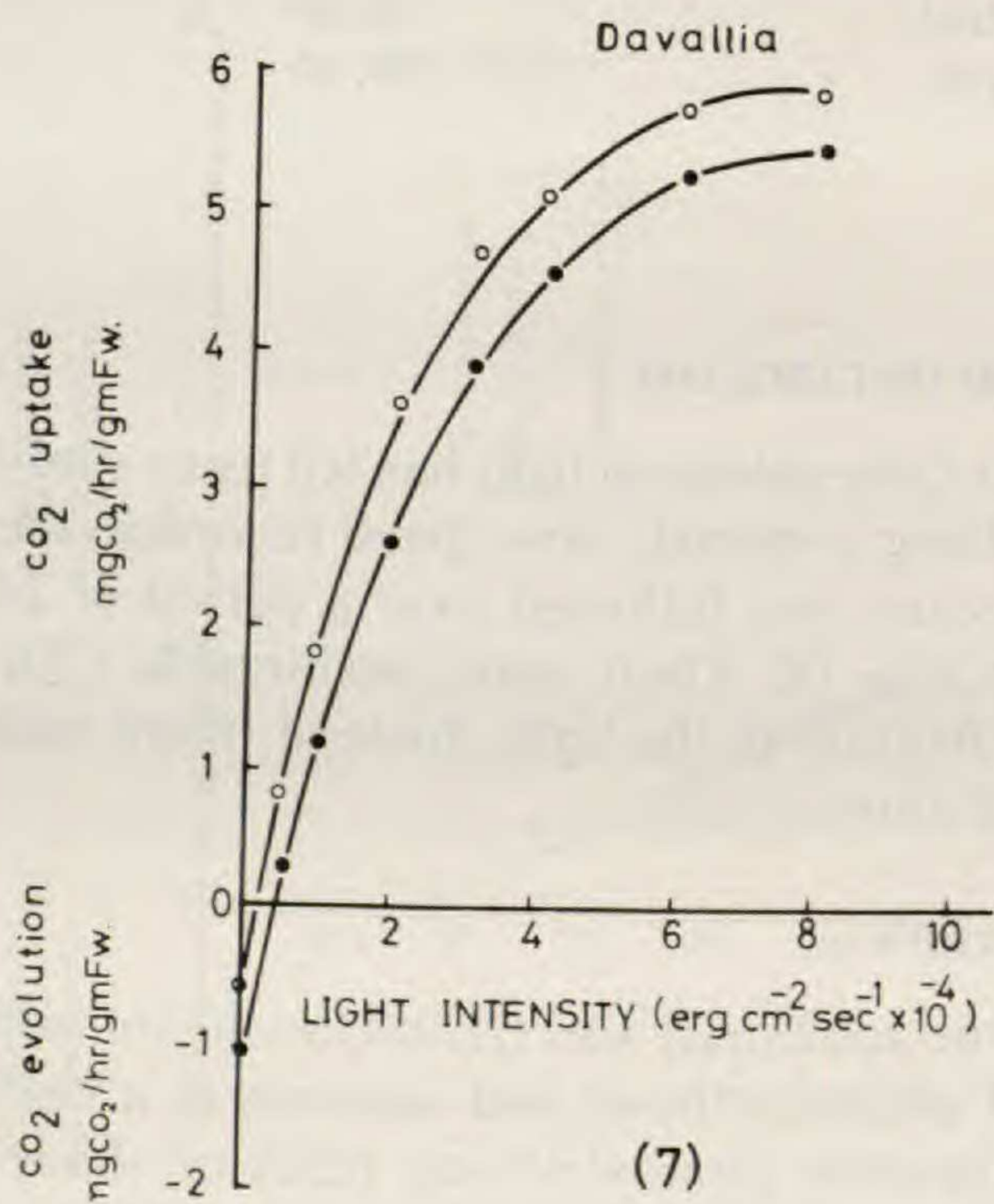
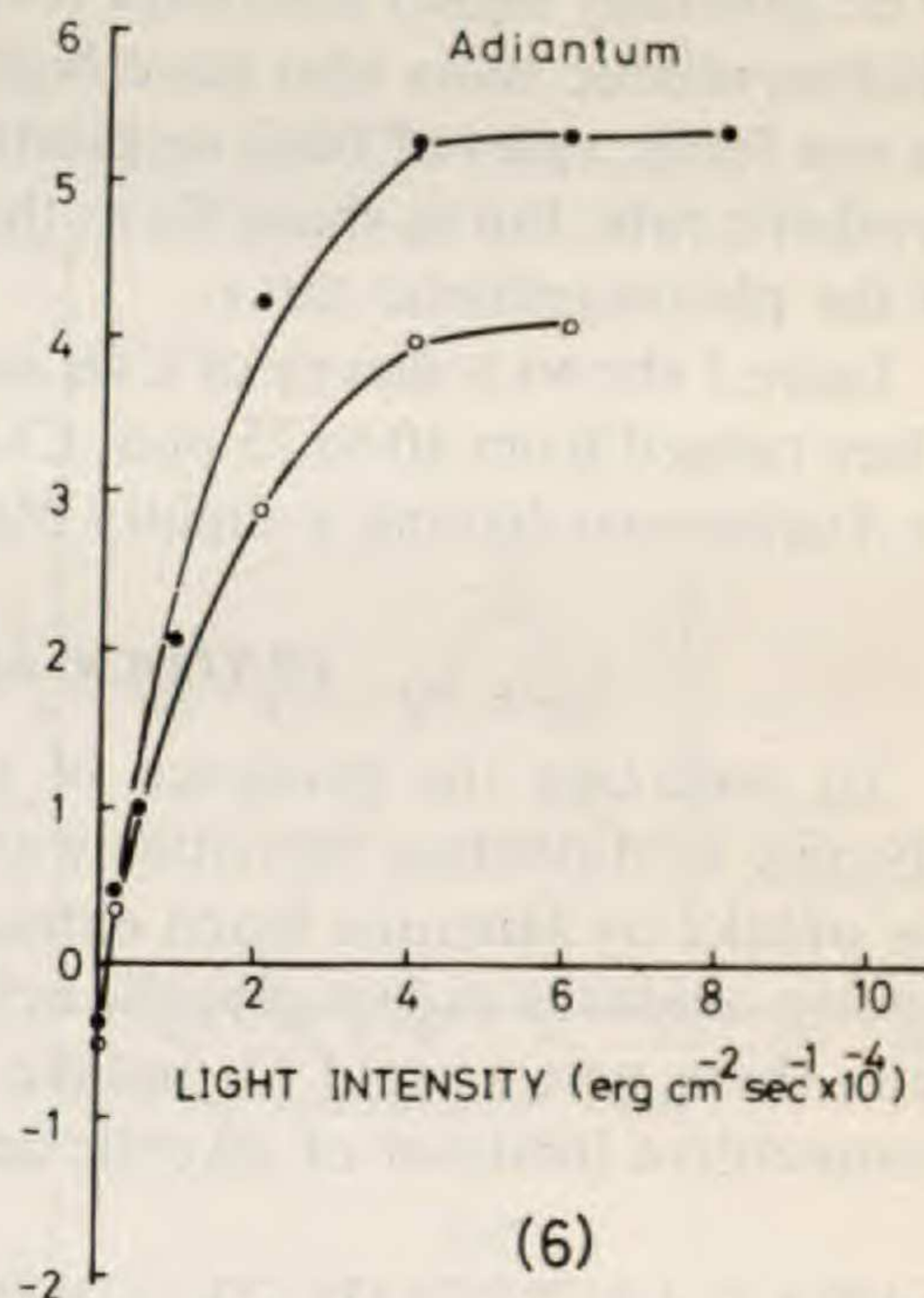
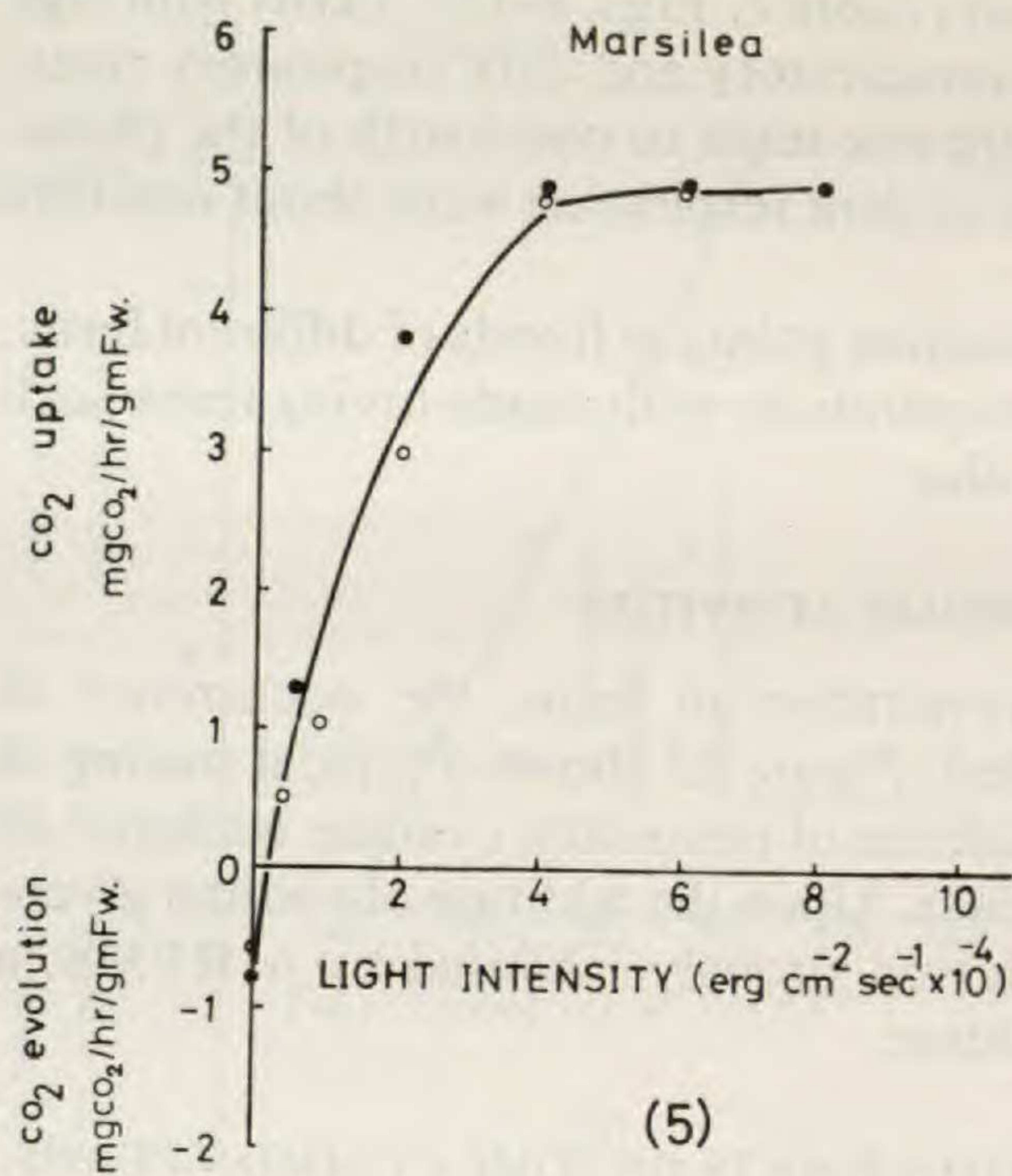
Photorespiration obtained by extrapolating the  $CO_2$  concentration versus apparent photosynthesis graph to zero  $CO_2$  concentration (Hew et al., 1969) was found





The effect of light intensity on apparent photosynthesis. FIG. 1. *Gleichenia*. FIG. 2. *Asplenium*. FIG. 3. *Nephrolepis*. FIG. 4. *Angiopteris*.





The effect of light intensity on apparent photosynthesis. FIG. 5. *Marsilea*. FIG. 6. *Adiantum*. FIG. 7. *Davallia*. FIG. 8. *Drymoglossum*.



to be generally higher than dark respiration (*Table 1; Figs. 9-12*). Ferns with high photosynthetic rates also have high photorespiratory and dark respiratory rates. In sun ferns, rates of dark respiration were one-tenth to one-fourth of the photosynthetic rate, but in shade ferns the rates of dark respiration were about one-fifth of the photosynthetic rates.

*Table 2* shows a survey of CO<sub>2</sub> compensation points in fronds of different ferns. They ranged from 40 to 75 ppm CO<sub>2</sub> concentration, with shade-loving ferns such as *Angiopteris* having a slightly higher value.

#### GLYCOLIC ACID OXIDASE ACTIVITIES

To ascertain the presence of photorespiration in ferns, the occurrence of glycolic acid oxidase activities was studied. *Figure 13* shows a typical tracing of O<sub>2</sub> uptake by *Marsilea* frond extract. Addition of potassium cyanide inhibited all known oxidases except glycolic acid oxidase. Upon the addition of sodium glycylate, there was a rapid O<sub>2</sub> uptake which was strongly inhibited by  $\alpha$  HPMS, a competitive inhibitor of glycolic acid oxidase.

TABLE 2. A SURVEY OF CO<sub>2</sub> COMPENSATION POINTS OF SOME COMMON FERNS.

<i>Species</i>	<i>Exposure &amp; Habitat</i>	<i>CO<sub>2</sub> compensation point (ppm)</i>
<i>Gleichenia linearis</i>	Sun, terrestrial	40, 47 <sup>1</sup>
<i>Nephrolepis biserrata</i>	Sun, terrestrial	52, 48
<i>Adiantum philippense</i>	Shade, terrestrial	43, 46
<i>Marsilea crenata</i>	Sun, aquatic	50, 51
<i>Angiopteris evecta</i>	Shade, terrestrial	70, 61
<i>Asplenium nidus</i>	Shade, epiphytic	69, 60

<sup>1</sup>Two replicates.

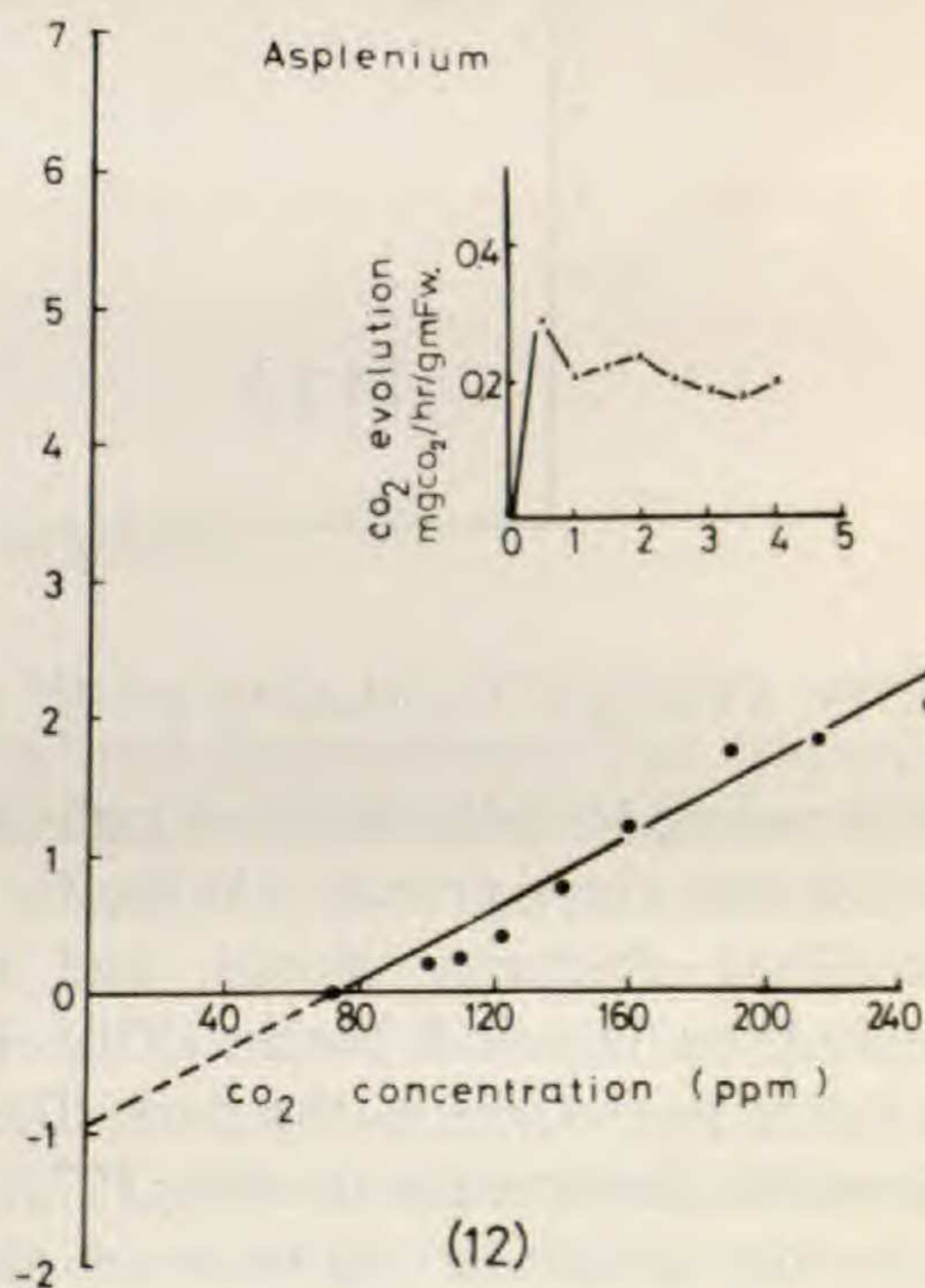
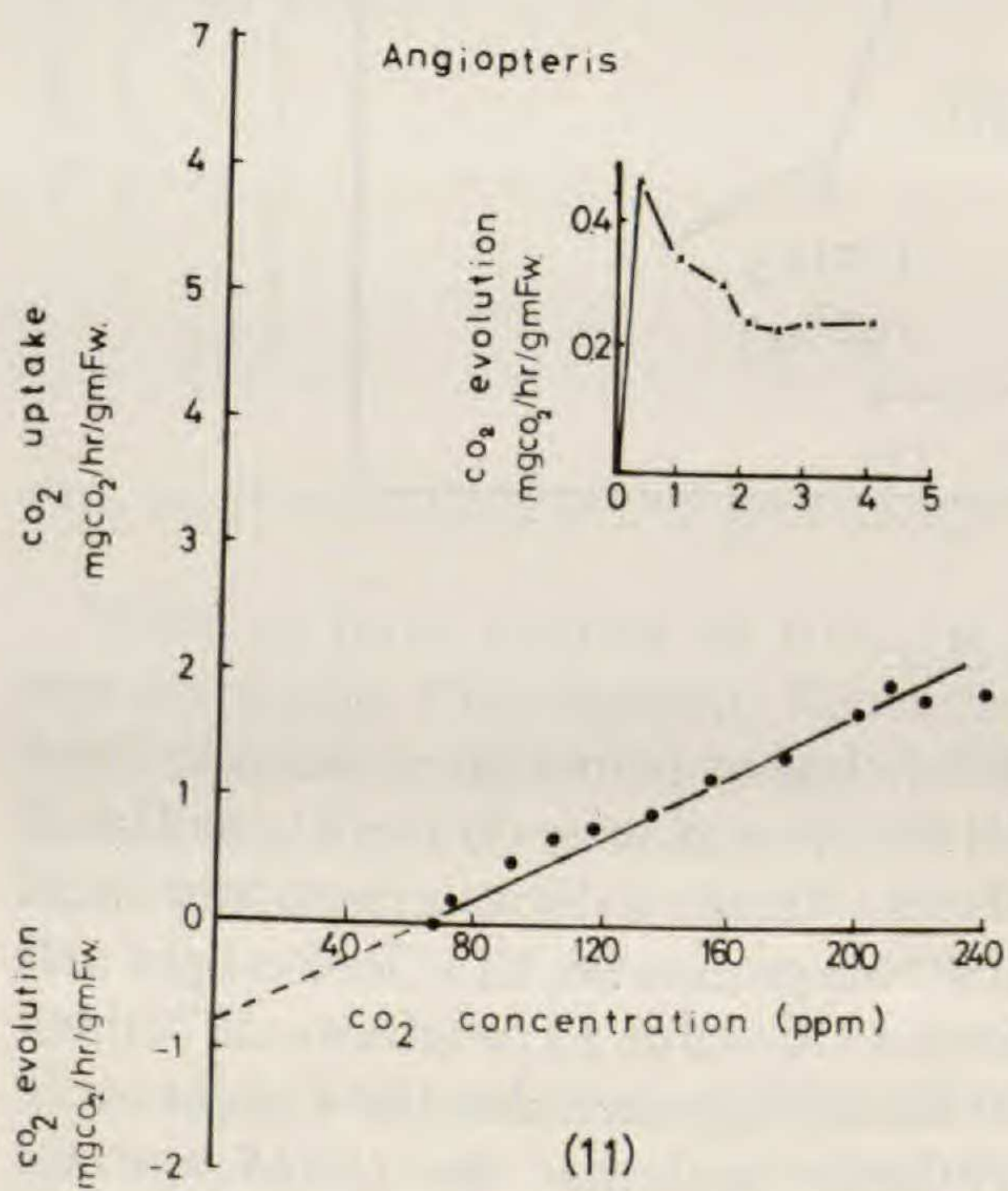
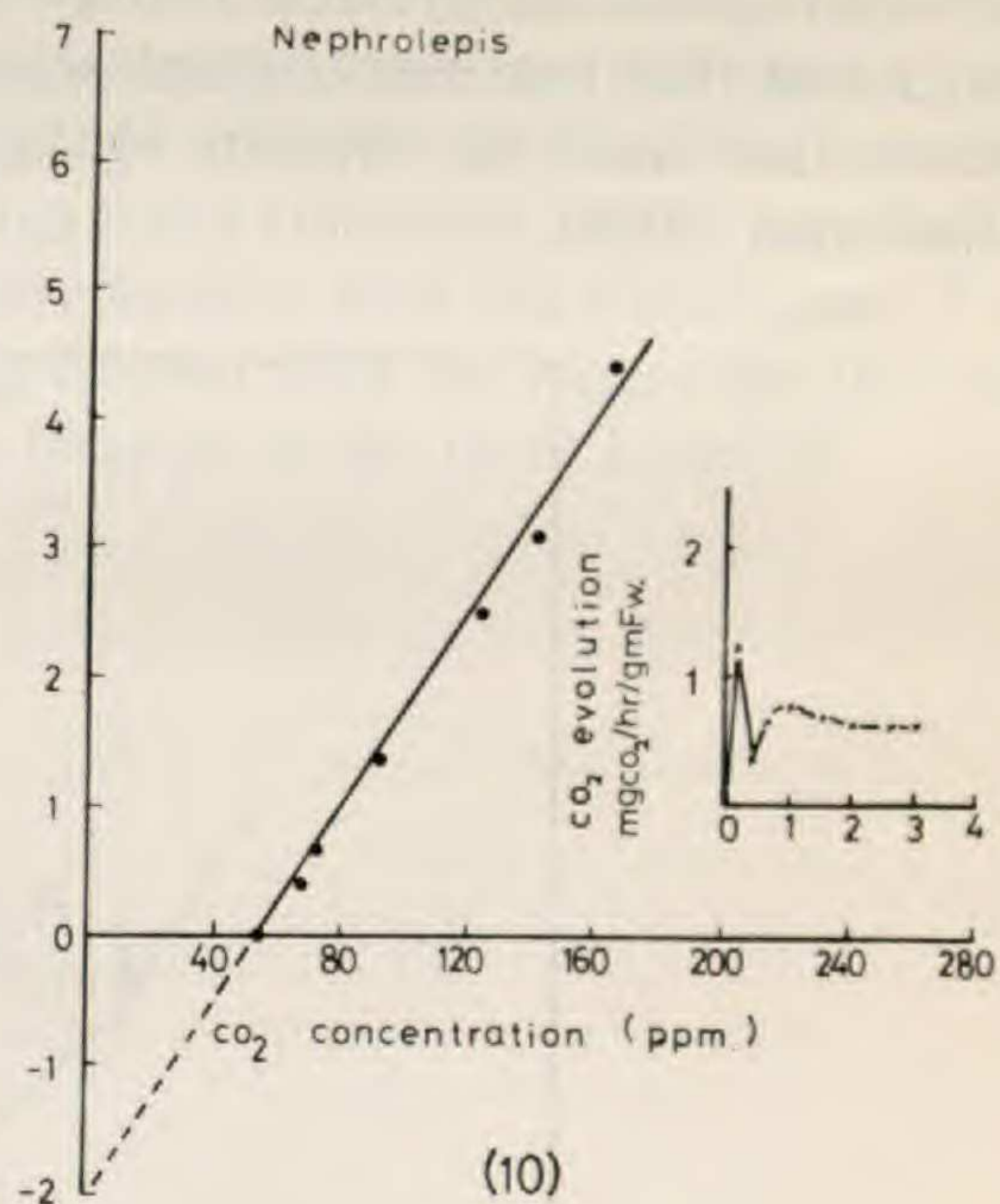
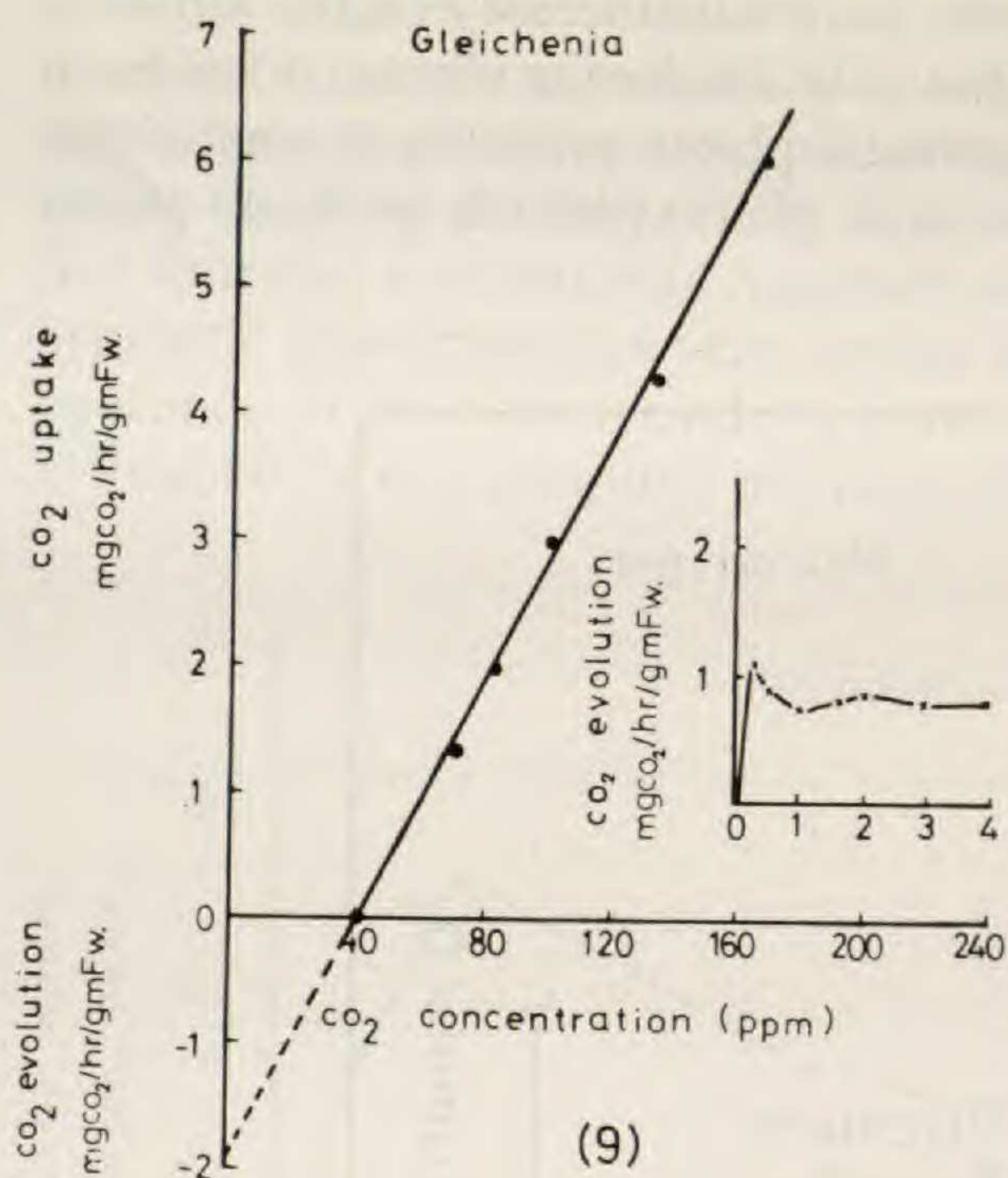
#### CRASSULEAN ACID METABOLISM

The fact that *Drymoglossum* showed no CO<sub>2</sub> uptake in light has led us to study the diurnal changes of its CO<sub>2</sub> fixation. Using a special, slow speed recorder, the CO<sub>2</sub> fixation and evolution of *Drymoglossum* was followed over a period of 24 hours. The results obtained are shown in *Fig. 14*. There was considerable CO<sub>2</sub> fixation in the dark, but little or no CO<sub>2</sub> fixation in the light. Instead, there was CO<sub>2</sub> evolution during the initial period of illumination.

#### DISCUSSION

The present investigation shows that the terrestrial sun ferns *Gleichenia* and *Nephrolepis* have high rates of apparent photosynthesis and saturate at a light intensity of  $1 \times 10^5$  erg cm<sup>-2</sup> sec<sup>-1</sup>. Apparent photosynthetic rates of shade-loving ferns, such as *Asplenium* and *Angiopteris*, were only one-sixth of that in sun ferns, and saturated at a very low light intensity. These results agree with the finding of sun and shade species of higher plants reported previously (Rabinowitch, 1951).





The effect of CO<sub>2</sub> concentration on apparent photosynthesis. FIG. 9. *Gleichenia*. FIG. 10. *Nephrolepis*. FIG. 11. *Angiopteris*. FIG. 12. *Asplenium*.



Because plants occurring in sunny habitats have considerably higher ribulose 1-5-diphosphate carboxylase activity than that of shade-loving plants, it has been concluded that low carboxylase activity in shade plants probably is one of the factors that limits the capacity for light-saturated photosynthesis in shade plants (Björkman, 1968).

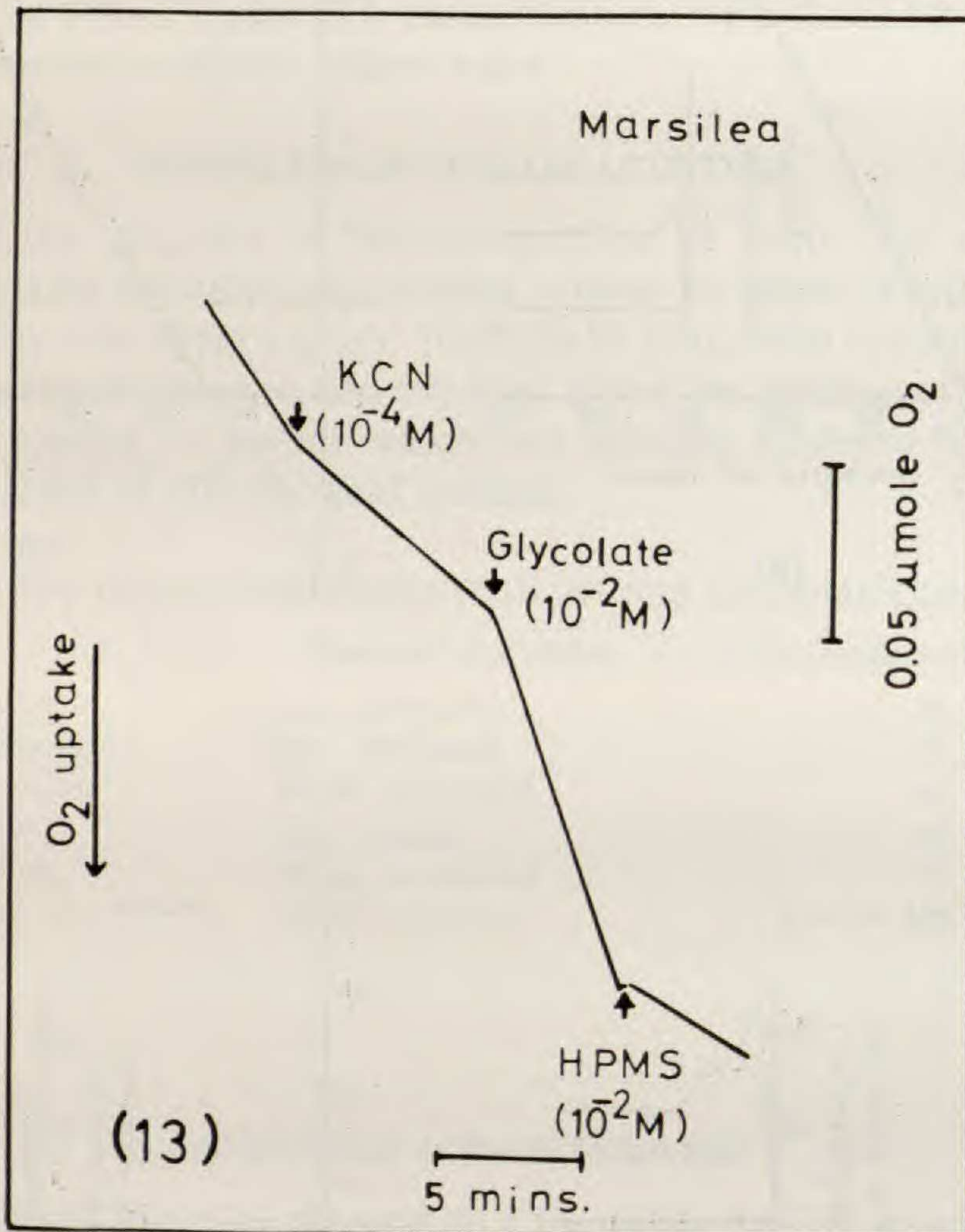


FIG. 13. A tracing of O<sub>2</sub> uptake by glycolic acid oxidase.

According to their mode of carbon fixation, higher plants have recently been divided into three groups: (1) the C<sub>3</sub> or Calvin cycle plants, (2) the C<sub>4</sub> or Hatch and Slack Pathway plants, and (3) plants which exhibit crassulacean acid metabolism (Hatch & Slack, 1970). The first two groups fix CO<sub>2</sub> in the light and the latter fixes CO<sub>2</sub> in the dark. Based on δ<sup>13</sup>C value (Troughton, 1971) and taxonomic distribution (Evans, 1971), it has been suggested that the Calvin cycle is a more primitive pathway of photosynthesis, and that the CAM and C<sub>4</sub> pathways are recent addenda to the Calvin cycle. All plants, however, rely on the Calvin cycle for the ultimate step in CO<sub>2</sub> fixation; the CAM and C<sub>4</sub> pathways operate essentially as mechanisms for prefixing and concentrating CO<sub>2</sub> (Evans, 1971).



In our present investigation, all the ferns studied except *Drymoglossum* show prominent postillumination  $\text{CO}_2$  outbursts; high  $\text{CO}_2$  compensation points, and high photorespiratory rates; all these are typical characteristics of Calvin cycle plants (Jackson & Volk, 1970). It is interesting to note that *Angiopteris*, a primitive fern that is commonly regarded as a living fossil (Holttum, 1954), has photosynthetic characteristics very similar to a more modern fern like *Gleichenia*. The operation of the Calvin cycle in ferns is in agreement with the suggestion that the Calvin cycle is a primitive pathway of  $\text{CO}_2$  fixation in the plant kingdom.

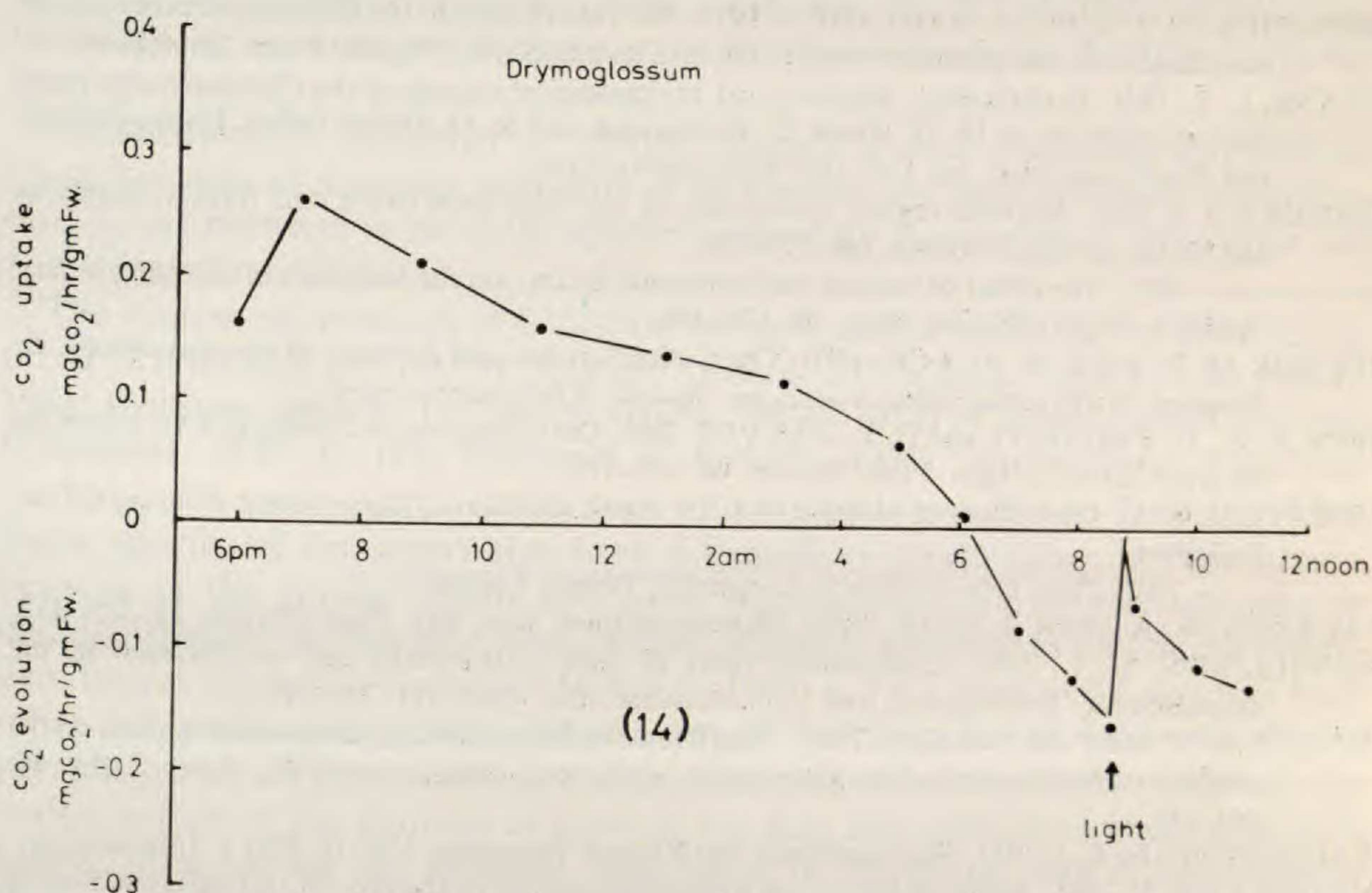


FIG. 14. Diurnal change of  $\text{CO}_2$  gas exchange in *Drymoglossum*.

While all ferns studied fix  $\text{CO}_2$  via the Calvin cycle, *Drymoglossum* exhibits non-autotrophic  $\text{CO}_2$  fixation. Recently, it has been demonstrated that crassulean acid metabolism is positively associated with succulent habit (McWilliams, 1970). It is generally believed that CAM is an adaptation to a xeric environment. Epiphytic habitats in tropical rain forests may be extremely xeric, indeed (Richards, 1952). However, it should be pointed out that although the epiphytic habitat may be xeric, it does not necessarily follow that an epiphyte would exhibit Crassulean acid metabolism. The difference in  $\text{CO}_2$  fixation patterns between the two epiphytic ferns *Asplenium* and *Drymoglossum* is, therefore, worth noting. *Drymoglossum* fronds are succulent but *Asplenium* is not. Presumably, *Asplenium* is not under a strong xeric stress. This is probably attributed to the elaborate root system of *Asplenium*, which acts as a trap in retaining water and other organic materials (Holttum, 1957).



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